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14. ABSTRACT- Parkinson's disease (PD) is a common chronic neurodegenerative disorder affecting one per one hundred adults over age 50. The underlying cause(s) of PD remain unknown. Dr. Cohen, the original recipient of the grant, titled "Parkinson's Disease; the link between monoamine oxidase (MAO) and mitochondrial respiration", proposed that the dopamine-metabolizing enzyme monoamine oxidase could potentially injure dopaminergic neurons; mediated initially by the formation of hydrogen peroxide and subsequently its conversion to water at the expense of formation of glutathione disulfide, catalyzed by glutathione peroxidase. The formation of the electrophilic glutathione disulfide in principle, and subsequently demonstrated experimentally, is able to subsequently form protein-cysteinyl-thiol-glutathione mixed disulfides (Pro-Cys-S-S-Glu). As Dr. Cohen was an expert in studying mitochondrial function, he further proposed that Pro-Cys-S-S-Glu mixed disulfides could lead to inhibition of electron transport chain function as a result of chemical modification of critical protein thiols in the mitochondrial electron transport chain. Depriving dopaminergic neurons of energy as a result of modification of critically-important thiol-moieties in mitochondria could secondarily lead to neuronal injury and death, providing a potential pathway contributing to the development of Parkinson's Disease. Whereas Dr Cohen focused on monoamine oxidase, my colleagues and I characterized a second mitochondrial inhibitory pathway involving catechol-oxidative pathways independent of MAO.

15. SUBJECT TERMS- A) Mitochondrial electron transport chain inhibition by a) dopamine b) dopamine-metabolites c) dopamine-quinones B) Inhibition of mitochondrial electron transport chain by glutathione-protein-thiol mixed-disulfides

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INTRODUCTION

In regards to neurologic disorders, Parkinson's Disease (PD) is one of the most common chronic neurodegenerative disorders, affecting about 1 in 100 adults age 50 and older. PD is characterized clinically by signs and symptoms that include tremor, rigidity and gait instability with less common symptoms including dementia and autonomic instability. The underlying brain pathology observed in PD patients is neurodegeneration of nigral-striatal dopaminergic pathways involving the striatum and substantia nigra. As with most chronic neurodegenerative diseases the exact biochemical, molecular and biologic mechanisms underlying the development of PD remains unanswered. Broadly considered, postulated etiologies of PD include genetic causes, exposure to metals and environmental/industrial zenobiotics and oxidative stress, among others.

The main neuroanatomic pathology seen in patients with PD is the relative selective loss of dopaminergic neurons in the substantia nigra. The precise reason(s) why dopaminergic neurons are selectively affected remains a mystery. The original recipient of the army grant was Dr Gerald Cohen, PhD, and his study was titled "Parkinson's disease: The link between monoamine oxidase and mitochondrial respiration". Dr. Cohen had extensive experience and research expertise in the field of mitochondrial dysfunction. His grant proposed examining potential biochemical and basic chemical mechanisms of dopaminergic neuronal injury and death, that might contribute to the development of PD. Dr. Cohen applied his expertise to mitochondrial studies to examine the role of oxidative stress and dopamine metabolism, to explore possible contributory mechanisms leading to injury and death of dopaminergic neurons; and therefore by implication, its potential relevance to the development of PD.

Oxidative damage is a broadly defined term used for describing a related group of chemical reactions, both organic and inorganic, involved in oxidation-reduction (redox) chemistry. More generally, oxidative damage is frequently used in describing reactions involving molecular oxygen or oxygen containing species such as hydrogen peroxide (H_2O_2). In this regard oxidation can involve the insertion of oxygen into molecules directly or removal of electrons without oxygen insertion (i.e. oxidation of metals such as ferrous iron to ferric iron). Consequently, oxidation can occur in the absence of oxygen.

BODY OF INVESTIGATON

Dr. Cohen's investigation was titled "Parkinson's disease; The link between monoamine oxidase and mitochondrial respiration". Dr. Cohen forwarded the following hypothesis: Monoamine oxidase (MAO) is a catecholamine metabolizing enzyme that biochemically converts monoamines such as dopamine into metabolites that are eventually excreted from the body. In the case of dopamine the monoamine is initially converted into the aldehyde DOPAL, and thereafter, into the carboxylic acid derivative DOPAC.

Figure 1 shown below illustrates the critical chemical reactions that led to Dr. Cohen's main aim of his investigation.

FIGURE 1. Critical reaction chemistry related to dopamine metabolism.

1) Dopamine (DA) + O_2 + H_2O → 3,4-dihydroxyacetalaldehyde (DOPAL) + H_2O_2 + NH_3

2) H_2O_2 + 2 glutathione (2 GSH) → Glutathione disulfide (GSSG) and 2 H_2O

3) GSSG and protein-thiol (Pr-SH) → Protein-glutathione mixed disulfide (Pro-S-S-G) + GSSG

Reaction 1): catalyzed by monoamine oxidase
Reaction 2): catalyzed by glutathione peroxidase
Reaction 3): ? enzymatic mediated

Dr. Cohen's main focus of his examination involved the demonstration that dopamine-associated metabolism to DOPAL, catalyzed by monoamine oxidase was associated initially with hydrogen peroxide formation with subsequent conversion of hydrogen peroxide to water, in the presence of GSH, forming glutathione-disulfide (GSSG). This reaction effectively converts chemically active hydrogen peroxide to the inactive species water. Dr. Cohen postulated that the oxidized and highly electrophilic GSSG could subsequently react with thiol-residues on protein cysteinyl-SH groups by nucleophilic substitution chemistry, resulting in the formation of protein cysteinyl-glutathione-protein mixed disulfides (Figure 1, reaction 3). As Dr Cohen's expertise was in mitochondrial function he observed and further postulated that formation of protein-glutathione mixed disulfides of proteins in the mitochondrial electron transport chain (ETC) led to a dose dependent inhibition of mitochondrial ETC function. Thus, the main hypothesis examined by Dr. Cohen was that MAO-dependent oxidative damination of dopamine led to the formation of protein-GSH mixed disulfides which demonstrated a dose dependent inhibitory response on the mitochondrial ETC in isolation rat brain mitochondria. In particular, as dopaminergic neurons are highly dependent on high levels of energy production from mitochondria, Dr Cohen posited that impairment of mitochondrial ETC function might affect survival of dopaminergic neurons, contributing to the development of Parkinson's disease.

KEY RESEARCH ACCOMPLISHMENTS

Figure 1 provides the basic hypothetical biochemical lattice that was built on by myself and my colleagues Dr. Gail Zeevalk, Associate Professor-Neurology Department/Neurosciences (UMDNJ-Robert Wood Johnson Medical School-Piscataway New Jersey), and Dr. Alpa Gautam (a PhD student at the time of the investigation). Dr. Cohen was the original principal investigator of the present grant. However, he unexpectedly passed away towards the completion of the latter part of the grant. Although I never directly collaborated with Dr. Cohen, our mutual collaboration with Dr W Nicklas PhD. of UMDNJ-Robert Wood Johnson Medical School, department of Neurology, where I completed my post-doctoral training was the underlying reason I was asked to complete the grant given the similar interests and overlap

of our work on mechanisms of mitochondrial dysfunction. I had not much personal contact with Dr. Cohen, but I felt confident that his project could be reasonably completed under my supervision. Additionally, Dr Gautam, as a graduate student in the laboratory of Dr. Zeevalk expanded further on Dr. Cohen's studies as a PhD graduate student as described later. In summary, Dr Cohen was able to initially establish the fundamental data supporting his hypothesis, and these findings were subsequently expanded on by Dr. Zeevalk and myself, thereafter with Dr Gautam during her PhD investigations.

REPORTABLE OUTCOMES: CUMULATIVE EXPANDED RESULTS:

The work of Drs. Zeevalk, Gautam and myself further confirmed and expanded the work of Dr Cohen. Dr Zeevalk and myself postulated that if Dr. Cohen's hypothesis was correct then deaminated catecholamines such as DOPAC(dopa-acetic acid) or DOPAL (dopa-aldehyde) should not cause any mitochondrial inhibition if the inhibition was exclusively a consequence of MAO-mediated oxidative deamination. However, our collective summarized results shown below, Table 1, showed that while dopamine could inhibit mitochondria in a dose-dependent fashion, the metabolites of dopamine including DOPAC were fairly potent inhibitors of mitochondrial ETC activity as well. In particular, DOPAC is not a substrate for MAO. Thus, these latter findings expanded on Dr. Cohen's hypothesis and this extended the scope of pathways responsible for mitochondrial inhibition of the ETC. In an effort to better clarify this seemingly different inhibitory mechanism we investigated the potential contribution of the catechol-moiety in dopamine and DOPAC, as another potential mediator of mitochondrial respiration inhibition. Also listed in Table 1 are hypothetical considerations for DOPAL and 2-O-methyl-DOPAC and 4-O-methyl-DOPAC. DOPAL was not commercially available.

Table 1. Inhibition of mitochondrial respiration by dopamine and DOPAC; and theoretical considerations for DOPAL and O-methyl-DOPAC. In testing inhibition by dopamine, studies were performed in the presence and absence of MAO A/B inhibitors. In agreement with Dr. Cohen's original studies our dopamine studies similarly demonstrated that inhibition of mitochondria by dopamine was partly contributed by monoamine oxidase activity (data not shown).

	Dopamine	DOPAL	DOPAC	O-methyl-DOPAC
Amine containing	YES	No	NO	NO
Catechol containing	YES	YES	YES	NO
mitochondrial electron-transport chain inhibition	YES	hypothetically YES	YES	hypothetically NO

SUMMARY OF EXPANDED RESULTS/REPORTABLE OUTCOMES (Drs. Zeevalk, Gautum and Gluck)

In addition to MAO-mediated mitochondrial electron transport chain inhibitionwe also explored

instead the potential role of catechol-mediated inhibition of mitochondrial electron transport function by dopamine and DOPAC. As DOPAC similarly inhibited electron transport chain function in brain mitochondria and that the inhibition could be partially attenuated in the presence of anti-oxidants, these findings suggestive an additional inhibitory pathway of electron transport independent of MAO-mediated mechanisms. Using peroxide neutralizing enzymes including catalase and found that inhibition by DOPAC could be partially attenuated, suggesting a role for peroxide formation as a contributing inhibitory pathway of mitochondrial electron transport chain function. As anti-oxidants we were able to further attenuate the inhibition as well we proposed that MAO-independent pathways of dopamine and DOPAC oxidation could similarly inhibit mitochondrial respiration. We believe that the inhibition of the electron transport chain was a result of free-radical mediated or reactive oxygen mediated pathways in the case of dopamine and DOPAC, and by inference, hypothetically, by free radical mediated mechanism with DOPAL as well. An alternate mechanism proposed by Hastings et al., previously, hypothesized that dopamine-quinones were also contributing to the inhibition of electron transport chain inhibition via formation of dopamine-protein cysteinyl-thiol adducts. As the catechol-ring is necessary for formation of the adduct, Dr. Gautam in her thesis also explored the potential for DOPAC to similarly form dopac-quinones as well. As she similarly demonstrated inhibition of electron transport chain activities via quinone formation as previously reported by Hastings et al., the Dr. Gautam further extended the work and demonstrated the formation of DOPAC-quinones as a probable contributory mechanism to electron transport chain inhibition.

Lastly, a note about the final component of doctoral research studies by Dr. Gautam and its relationship to Dr. Cohen's studies. Historically, Dr. Gautam was initially an undergraduate student working in my laboratory part-time. Upon completing her undergraduate degree at Hunter College, City University of New York (CUNY) Alpa went on to receive her PhD from the University of Medicine and Dentistry New Jersey-UMDNJ completing her doctoral work in the laboratory of Dr. Gail Zeevalk, who was my collaborator while we continued our mitochondrial studies. Therefore, Dr. Gautam represents a third generation of investigators (including Dr. Cohen) that furthered Dr. Cohen's studies. Dr. Gautam's work also expanded on Dr. Cohen's as she focused on the role of glutathione in contributing to potential pathways of mitochondrial dysfunction and inhibition of electron transport as well. Dr. Gautam's investigations examined the distribution of glutathione-protein mixed disulfides. She examined the distribution of the cysteinyl protein-glutathione mixed disulfides in various regions of rats, and postulated from her findings, that paradoxically and interestingly, that cysteinyl-protein-glutathione mixed disulfides may serve a role as a neuroprotective agent of critical cysteinyl-thiol residues in brain proteins. She proposed this hypothesis based on immunochemical studies in rat brain; and based on her data (not shown) the possibility that protein-cysteinyl conjugates may be transient, reversible and short-lived due to the chemical degradation of the disulfides by

glutathione reductase and glutaredoxin. As the conjugates between GSH and protein cysteinyl-thiols were reversible she proposed in the final section of her thesis that the transient formation of these conjugates may provide a short-lived protective chemical milieu protecting against oxidation of cysteinyl-protein thiols and the formation of their corresponding sulfates/sulfites.

Paradoxically. Dr. Cohen postulated that although glutathione by itself could provide anti-oxidant protection, protein thiol-glutathione mixed disulfideadducts could result in adverse effects, reducing electron transport function in mitochondria. Dr. Gautam's work suggested that the mixed-disulfides, under conditions of reversibility, could protect mitochondria against oxidative damage. Therefore, taken collectively it appears that in regards to the potential role of glutathione chemical modification of cysteinyl-containing proteins that GSH demonstrated a dual role; detrimentally in the studies based on Dr. Cohen who postulated cysteinyl modification could lead to inhibition of electron transport chain proteins in mitochondria, and potentially protective in nature in regards to Dr. Gautam's work, suggesting that the disulfides could serve as transient reversible blocking agents preventing cysteinyl oxidation mediated by mitochondrial generated free radicals.

CONCLUSIONS:

The findings briefly presented in these research summary results represent three generations of investigators as noted previously. In general, it appears that Dr. Cohen's fundamental hypothesis regarding the potential deleterious effects of dopamine on mitochondrial electron chain function, mediated by chemical modification of critical cysteinyl-thiol-protein moieties in the electron transport chain leading to glutathione-protein mixed disulfides, was reproducible and therefore in this author's view a reasonably correct model. The subsequent findings of Dr. Zeevalk and myselffurther indicated that MAO dependent as well as MAO-independent pathways could also potentially contribute to mitochondrial electron transport chain inhibition as well. Furthermore, metabolites of dopamine including DOPAC and hypothetically DOPAL are able to inhibit electron transport chain activity as well. Lastly, it appears from the work of Dr. Gautam that glutathione-cysteinyl protein mixed disulfides may serve at times a neuroprotective function, in contrast to the proposed deleterious effects originally proposed by Dr. Cohen.

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APPENDICES:

Separate attachments scanned into document file